Effect of Dietary Phosphorus, Phytase, and 25-Hydroxycholecalciferol on Broiler Chicken Bone Mineralization, Litter Phosphorus, and Processing Yields

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ABSTRACT Three floor pen experiments (Exp) were conducted to evaluate low nonphytin P (NPP) concentrations and the NPP sparing effect of phytase (PHY) and 25-hydroxycholecalciferol (25D) on bone mineralization, bone breaking during commercial processing, litter P, and water-soluble P (WSP) concentrations. Tested treatments (TRT) were control, National Research Council NPP; University of Maryland (UMD) NPP; UMD + PHY, UMD NPP reduced by 0.064% NPP + 600 U of PHY/kg; UMD + PHY + 25D, UMD NPP reduced by 0.090% NPP + 600 U of PHY and 70 μg of 25D/kg; control + PHY mimicked the industry practice of diets by 0.1% when PHY is added; and negative control with 90% UMD NPP concentrations. UMD + PHY and control + PHY diets contained 600 U of PHY/kg, and UMD + PHY + 25D contained 600 U of PHY + 70 μg of 25D/kg. Performance results were presented separately. After each Exp, litter P and WSP were determined, and bone measurements were obtained on 8 or 10 broilers per pen. Tested TRT did not affect broiler BW. Femur ash weight of broilers fed the UMD and UMD + PHY + 25D was lower in all Exp compared with that of broilers fed the control diet. Femur ash was similar for control and UMD + PHY broilers, yet averaged over all Exp, UMD + PHY broilers consumed 39% less NPP and required less NPP per gram of femur ash than those on the control (4.87 and 7.77 g of NPP/g of ash, Exp 3). At the end of Exp 3, broilers were processed in a commercial facility. Despite reductions in NPP intake and bone mineralization, no differences were observed in measurements of economic importance (parts lost, carcass yield, and incidence of broken bones). The P excretion per bird was lowest for birds fed the UMD + PHY + 25D diet followed by those fed the UMD + PHY and negative control diets (10.44, 12.00, and 13.78 g of P/bird, respectively) and were highest for those fed the control diet (19.55 g of P/bird). These results suggest that feeding diets low in P together with PHY and 25D will not affect performance or increase losses at processing while resulting in improved P retention and reductions in P and WSP excreted.

Key words: nonphytin phosphorus, phytase, bone mineralization, processing plant, litter phosphorus

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INTRODUCTION

Recent research suggests that National Research Council (1994) nonphytin P (**NPP**) recommendations for broiler chickens exceed requirements (Skinner et al., 1992a,b; Angel et al., 2000a,b; Ling et al., 2000; Waldroup et al., 2000; Yan et al., 2000; Dhandu and Angel, 2003; Angel et al., 2005b). Feed additives such as phytase (**PHY**) and 25-hydroxycholecalciferol (**25D**) have been shown to reduce the need for supplemental P by improving the use of dietary phytin P (Mitchell and Edwards, 1996a,b; Yan et al., 2000, 2001, 2003; Waldroup et al., 2000; Angel et al., 2001, 2005b). However, most of these studies have not focused on the effect of feeding lower dietary NPP concentrations, with or without feed additives, on processing losses as well as litter P and water-soluble P (**WSP**).

Commercial implementation of NPP requirements and full implementation of PHY (Kornegay et al., 1996; Angel et al., 2001; Yan et al., 2001) and 25D (Mitchell and Edwards, 1996a,b; Sebastian et al., 1996; Biehl and Baker, 1997; Zanini and Sazzad, 1999; Waldroup et al., 2000; Angel et al., 2001) efficacies have been limited in part because of the lack of information on the effects of these changes at catching and processing. Limited research has been conducted that focuses on the effect of P nutrition and resulting bone mineralization and how these correlate with catching and processing losses. Two papers have been published that partially address the issue of the impact of reduced dietary P concentrations on processing losses (Moran and Todd, 1994; Chen and Moran, 1995).

Bone ash has been used extensively as a criterion for evaluating P requirements because it is a more sensitive indicator of dietary P sufficiency than growth rate (Wal-

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droup et al., 2000; Yan et al., 2001; Dhandu and Angel, 2003). Research has shown that bone mineral content (**BMC**) as well as densitometry measurements, using dual-energy x-ray absorptiometry (**DXA**; Mitchell et al., 1997, 1998a,b; Angel et al., 2004), can be used to assess bone mineralization. This method allows for accurate determinations of whole body bone density and mineral content (Mitchell et al., 1997; Angel et al., 2004) while resulting in decreased labor and time investment as compared with the traditional bone ash methodology.

Decreasing dietary P, to concentrations that do not impair broiler performance, results in decreased litter P (Waldroup et al., 2000; Yan et al., 2000, 2001, 2003; Dhandu and Angel, 2003). Inclusion of PHY in poultry diets results in more of the phytic acid P present in the seed-based ingredients being available and thus absorbed by the animal, reducing the need for supplementation with inorganic dietary sources of NPP such as dicalcium phosphate, hence reducing both dietary (Waldroup et al., 2000; Yan et al., 2001; Applegate et al., 2003; Angel et al., 2005a) and litter (Waldroup et al., 2000; Saylor et al., 2001) P concentrations.

Controversy exists about the effects of the use of PHY on excreted concentrations of WSP and the ratio of WSP to P (Moore et al., 1998; Sims et al., 1999; DeLaune et al., 2004; Saylor et al., 2001). Phytase releases phosphate groups from the phytate molecule, making the released P available to the animal (Rapp et al., 2001). If dietary concentrations of inorganic P are not reduced appropriately when PHY is included, excretion of available P will increase, and thus the potential exists for the increases in excreted WSP as well as an increase in the proportion of P that is WSP (Angel et al., 2005a).

The objectives of the current research were to determine the effect of applying NPP requirements and the NPP-sparing effects of PHY and 25D that had previously been determined in battery cages under conditions simulating commercial broiler production on performance, bone mineral measurements, carcass yields, processing losses, and litter P and WSP concentrations. Broiler performance data from this series of studies have been reported previously (Angel et al., 2005b).

MATERIALS AND METHODS

All procedures used in the broiler chicken experiments (**Exp**) were approved by the University of Maryland Animal Care and Use Committee. Three floor pen Exp (Exp 1, 2, and 3) of similar design were conducted sequentially using male Ross 308 broiler chickens obtained from a commercial hatchery on the day of hatch. Fifty-six broiler chickens were randomly allocated to 55 pens (0.074 m^2/bird), and treatment (**TRT**) assignment to pens for the 3 Exp was identical. A 4-phase feeding program was used: starter (hatch to 18 d), grower (18 to 32 d), finisher (32 to 42 d), and withdrawal (42 to 49 d). Broiler chickens were fed corn-soybean-based diets formulated to meet or exceed National Research Council (1994) nutrient recommendations, except for NPP and Ca. Basal diet ingredient and nutrient (formulated and analyzed) compositions and dietary TRT details are provided in Angel et al. (2005b). Briefly, 6 dietary TRT were tested: control, National Research Council (NRC; 1994) NPP recommendations; University of Maryland (**UMD**) NPP recommendations (Angel et al., 2000a,b, 2001; Dhandu and Angel, 2003); UMD + PHY, UMD NPP concentrations reduced by 0.064% NPP plus 600 U (1 U of PHY is defined as the amount of enzyme required to liberate 1 μmol of inorganic P from 1.5 m*M* Na phytate at pH 5.5 and 37°C; Engelen et al., 1994) of PHY/kg of diet; UMD + PHY + 25D, UMD NPP concentrations reduced by 0.090% NPP plus 600 U of PHY and 70 μg of 25D/kg of diet; control + PHY, mimicked industry practice of reducing NRC (1994) concentrations by 0.1% when PHY is added; and negative control (**NC**) that had 90% UMD NPP concentrations (Table 1). The NPP concentrations used for the starter, grower, finisher, and withdrawal phases for the NRC and UMD diets were 0.45 and 0.45, 0.35 and 0.31, 0.35 and 0.23, and 0.30 and 0.18%, respectively. Dietary NPP differences were obtained through the use of different concentrations of monocalcium phosphate. Each TRT was fed to 9 replicate pens, except the NC TRT, which was fed to 10 pens.

All PHY-containing diets were formulated to contain 600 U of PHY from Ronozyme P-CT (DSM Nutritional Products, Basel, Switzerland)/kg of diet after pelleting. Detailed mixing and pelleting information is provided in Angel et al. (2005b). Hy-D (DSM Nutritional Products) was added to 25D-containing diets to attain an activity of 70 μg of 25D/kg of diet. The NPP concentrations in the NC diets in the finisher and withdrawal phases of Exp 3 were modified compared with those of Exp 1 and 2 to be 70 and 56% of the UMD NPP concentrations, respectively, instead of 90%, as in Exp 1 and 2. This change was made because there was no detectable performance effect in broilers fed the NC diets in Exp 1 and 2; thus, the NC was not considered to be a true NC. The reductions in NPP vs. Exp 1 and 2 were chosen based on the results of previous battery pen work (Angel et al., 2000a,b). Formulated and analyzed concentrations of dietary NPP, Ca, PHY, and 25D for the different feeding phases of each Exp are shown in Table 1. Analytical details are provided in Angel et al. (2005b).

At the end of each Exp (49 d), 10 (Exp 1 and 3) or 8 birds (Exp 2) per pen were randomly selected, weighed, killed by cervical dislocation, and frozen until scanned [whole body scan for bone mineral density (**BMD**) and **BMC** using DXA (Lunar DPX-L, Lunar Corp., Madison, WI (Mitchell et al., 1997)]. Tibia mineral density (**TMD**) and tibia mineral content (**TMC**) were measured from the total body scans using a region-of-interest analysis that included the right (46%) or left (54%) tibia. Choice of tibia (right or left) was dictated by clarity of scan and targeting a no-side bias (50:50) analysis. Selection of the right or left tibia for analysis was random unless one of the bones was eliminated due to the interference by other bones or poor alignment relative to the scan plane. After DXA analyses, the right tibia and femur were excised

Table 1. Formulated (Frm), determined (Det)[1] nonphytin P (NPP) and analyzed phytase (PHY) dietary concentrations in the starter (hatch to 18 d), grower (18 to 32 d), finisher (32 to 42 d), and withdrawal (42 to 49 d) phases, normalized to 90% dry matter, experiment (Exp) 1, 2, and 3[2]

	Starter				Grower				Finisher				Withdrawal			
		Det				Det				Det				Det		
		Exp				Exp				Exp				Exp		
Treatment[3]	Frm	1	2	3	Frm	1	2	3	Frm	1	2	3	Frm	1	2	3
Diet (%)																
Control	0.45	0.38	0.43	0.45	0.35	0.30	0.36	0.32	0.35	0.35	0.37	0.33	0.30	0.29	0.29	0.31
UMD	0.45	0.41	0.46	0.45	0.31	0.29	0.34	0.28	0.23	0.23	0.22	0.21	0.18	0.18	0.15	0.18
UMD + PHY	0.39	0.37	0.39	0.41	0.25	0.23	0.29	0.22	0.17	0.22	0.14	0.17	0.12	0.13	0.11	0.13
UMD + PHY + 25D	0.36	0.33	0.32	0.27	0.23	0.23	0.24	0.18	0.14	0.17	0.12	0.11	0.13	0.14	0.11	0.14
Control + PHY	0.35	0.34	0.33	0.37	0.25	0.28	0.28	0.23	0.25	0.25	0.26	0.25	0.20	0.20	0.18	0.24
NC	0.41	0.38	0.40	0.42	0.28	0.28	0.31	0.25	0.21[4]	0.19	0.19	0.13	0.16[4]	0.15	0.14	0.11
Analyzed PHY activity (U/kg of diet)[5]																
Control		32	162	0		17	103	75		71	99	12		72	50	19
UMD		23	73	0		0	90	50		67	58	24		56	46	21
UMD + PHY		683	708	625		448	677	646		640	563	633		546	614	642
UMD + PHY + 25D[6]		552	683	516		480	661	684		655	641	535		641	629	672
Control + PHY		518	765	730		470	685	733		687	594	596		94	640	676
NC		29	74	31		9	105	31		69	49	29		58	78	16

[1]Nonphytin P was determined by subtracting analyzed phytin P (PP) from analyzed P. Analyzed percentage of PP (mean ± SD) in the starter, grower, finisher, and withdrawal diets was 0.30 ± 0.02, 0.28 ± 0.01, 0.26 ± 0.03, and 0.26 ± 0.01, respectively, in Exp 1; 0.30 ± 0.01, 0.26 ± 0.02, 0.26 ± 0.01, and 0.27 ± 0.03, respectively, in Exp 2; and 0.27 ± 0.01, 0.28 ± 0.04, 0.28 ± 0.02, and 0.24 ± 0.02, respectively, in Exp 3.

[2]Formulated Ca (%) in the starter, grower, finisher, and withdrawal phases was 0.91, 0.81, 0.71, and 0.61, respectively, for all 3 Exp. Analyzed Ca (mean ± SD) in the 4 phases was 0.93 ± 0.02, 0.66 ± 0.05, 0.70 ± 0.1, and 0.59 ± 0.04, respectively, in Exp 1; 0.92 ± 0.01, 0.80 ± 0.08, 0.65 ± 0.07, and 0.57 ± 0.06, respectively, in Exp 2; and 0.95 ± 0.02, 0.84 ± 0.07, 0.69 ± 0.06, and 0.62 ± 0.04, respectively, in Exp 3.

[3]National Research Council (NRC) NPP recommendations; University of Maryland (UMD) NPP recommendations; UMD + PHY, UMD NPP concentrations reduced by 0.064% NPP plus 600 units (U) of PHY/kg of diet; UMD + PHY + 25-hydroxycholecalciferol (25D), UMD NPP concentrations reduced by 0.090% NPP plus 600 U of PHY and 70 μg of 25D/kg of diet; control + PHY, mimicked the industry practice of reducing NRC concentrations by 0.1% when PHY is added; and negative control (NC) that had 90% UMD NPP concentrations, except in the finisher and withdrawal phases of Exp 3.

[4]Results from Exp 1 and 2 showed that NPP concentrations in finisher and withdrawal phases were not sufficiently low in the NC, therefore NPP concentrations were reduced to 0.16 and 0.10% in Exp 3 vs. 0.21 and 0.16% in Exp 1 and 2 in the finisher and withdrawal phases, respectively.

[5]Formulated phytase activity in pelleted diets containing phytase was 600 U/kg of diet. One phytase U is defined as the amount of enzyme required to liberate 1 μmol of inorganic P from 1.5 m*M* of Na phytate at pH 5.5 and 37°C.

[6]Diets with 25D were formulated to contain 70 μg of 25D/kg of diet for all the phases. The analyzed concentrations (μg/kg of diet) in starter, grower, finisher and withdrawal phases were 79, 62, 72, and 71, respectively, in Exp 1; 55, 56, 80, and 63, respectively, in Exp 2; and 52, 89, 94, and 93, respectively, in Exp 3.

from each bird, all tissue along with the cartilage cap was removed, and dry defatted bone and ash weight, as well as ash percentage, were determined (Association of Official Analytical Chemists, 1990, method 972.15).

Processing Study

At the end of Exp 3, an additional 22 birds per pen were randomly selected, weighed, and wing-banded to maintain bird identity in the subsequent processing component of this study. These 22 birds per pen were considered as an experimental unit for the processing work. After the birds were wing-banded and weighed, they were placed in one common area within the broiler house, with only water available (feed withdrawal period) to better simulate whole-house catching and to maximize mixing of birds from different pens and TRT, thus minimizing catching pen effects. A commercial catching crew caught the wing-banded birds and transported them to a commercial processing plant 56 km away. Birds were processed (5 to 7 h after feed withdrawal) at the end of a regular shift in the processing plant to avoid mixing of experimental with commercial broilers. The line speed was 93 birds per minute. Carcasses were removed from the line just before entry into the chill tank. Broiler chickens were subjected to standard processing procedures [stunning (12 V and 0.2 mA for 6 s), bleeding, 3-stage scalding, picking (models Meyn JM64 and Linco D-22E, Davis Poultry Equipment, Dawsonville, GA), mechanical evisceration (model AE3, Johnson Food Equipment, Kansas City, KS), and normal inspection]. Plant as well as federal quality-control personnel inspected all birds for defects. Plant personnel condemned whole carcasses and removed parts for discarding, employing normal commercial standards. The only exception was that the wing-banded wing, if defective, was not removed so that bird identification would not be lost. If the wing was defective (broken or heavily bruised), it was noted upon inspection, and broken wings were considered to have been removed during normal processing and inspection for calculating yield.

Hot carcass weight was recorded and used to determine dressing percentage. The hot carcasses were examined for bruises by area [breast, legs (thigh and drumstick), and wings] and for broken legs (thigh and drumstick) and wings. Plant personnel then deboned carcasses to obtain breast meat (pectoralis major and minor) with skin, legs (thigh and drumstick), wings, and barrel (rib cage and back bone). All parts were saved and weighed to determine percentage of parts yield. Right tibia and femur from all processed birds were then removed as previously described, and dry defatted ash weight and ash percentage were determined (Association of Official Analytical Chemists, 1990, method 972.15).

Litter Study

Fresh pine shavings were weighed into each pen at the start of Exp 1, and a composite sample was taken for moisture, P, and WSP analysis. Between Exp, a composite litter sample was taken from each pen. Litter was sampled at 6 locations within each pen, with sampling occurring to the depth of the litter at each sample point. Litter samples from the 6 locations were pooled, mixed, and a composite sample (approximately 250 g) was taken; the remaining composite litter was returned immediately to the appropriate pen. After sampling, the litter in each pen was thoroughly mixed, which resulted in the cake being ground and mixed into the litter. There was no addition of fresh litter among flocks. At the end of Exp 3, all litter from each pen was removed, weighed, mixed, and sampled. Litter was ground to pass through a 1-mm screen and analyzed for moisture (Association of Official Analytical Chemists, 1990, method 967.03), P (Heinonen and Lahti, 1981) and WSP (Self-Davis and Moore, 2000).

Statistical Analysis

The experimental design was a randomized complete block with unequal replication. Pen was the experimental unit. The percentages (bruised or broken back, breast, wings, and legs) were transformed (arcsine square root) to meet the assumptions of ANOVA (Sokal and Rohlf, 1996). Data presented in tables are the arcsine mean values that were back transformed. If the overall effect of TRT was significant ($P \leq 0.05$) in the model, then differences between individual TRT means were separated by least significant difference test (Sokal and Rohlf, 1996) using SAS software (Statistical Analysis System, 2000). A probability of $P \leq 0.05$ was considered significant.

RESULTS

There were no differences in 49-d BW of the 10 or 8 broiler chickens per pen that were randomly sampled for bone mineral measurement in any of the Exp (Table 2). Detailed performance results for these Exp are given in Angel et al. (2005b). Regardless of Exp, hatch to 49-d NPP intake (**NPPI**) was greatest in birds fed the control diets, followed by those fed the control + PHY and UMD diets and least for those fed the UMD + PHY and UMD + PHY + 25D diets (Table 2). Dry tibia and femur weight was similar for all TRT except in Exp 1 in which birds fed the NC diets had lower dry tibia and femur weight than those on the control diets (Table 2). Femur ash weight of broilers fed the control diets was greater than that of broilers fed the UMD diets but not different from those fed UMD + PHY diets. There was no difference in either femur or tibia dry weight, ash weight, or ash percentage of broilers fed the UMD + PHY or control + PHY diets, yet the ratio of NPPI to femur or tibia weight or ash weight of broilers fed the UMD + PHY diet was lower than that of birds fed the control + PHY diets, and the lowest ratio was in broilers fed the UMD + PHY + 25D diets. The greatest NPPI per the femur, tibia dry weight, or ash weight was for broilers fed the control diets and least for those fed the UMD + PHY + 25D diets (Table 2). The *F*-ratio of tibia weight, ash weight, and ash percentage data were

Table 2. Body weight, nonphytin P (NPP) intake, and femur and tibia measurements in 49-d-old broilers, experiment (Exp) 1, 2, and 3

			Femur[3]			NPPI per femur[4]		Tibia[3]			NPPI per tibia[4]	
Treatment[1]	BW (g)	NPPI[2] (g)	Weight (g)	Ash (g)	Ash (%)	Weight (g/g)	Ash (g/g)	Weight (g)	Ash (g)	Ash (%)	Weight (g/g)	Ash (g/g)
Exp 1												
Control	2,893[5]	16.0[a]	4.70[a]	2.46[a]	52.34[a]	3.42[a]	6.53[a]	6.32[a]	3.38[a]	53.44	2.54[a]	4.76[a]
UMD	2,870	12.5[b]	4.55[ab]	2.31[cd]	50.57[c]	2.76[b]	5.45[b]	6.01[bc]	3.19[ab]	52.98	2.08[b]	3.93[b]
UMD + PHY	2,896	10.4[d]	4.69[a]	2.38[abc]	50.79[bc]	2.23[e]	4.39[d]	6.21[ab]	3.28[ab]	52.87	1.68[d]	3.18[d]
UMD + PHY + 25D	2,868	9.6[e]	4.77[a]	2.35[bcd]	49.21[d]	2.02[f]	4.11[e]	6.09[abc]	3.21[ab]	52.75	1.58[e]	3.00[e]
Control + PHY	2,894	12.3[b]	4.66[ab]	2.41[ab]	51.73[ab]	2.64[c]	5.11[c]	6.25[ab]	3.31[ab]	52.96	1.97[c]	3.72[c]
NC	2,835	11.2[c]	4.47[b]	2.25[d]	50.39[c]	2.52[d]	5.00[c]	5.89[c]	3.09[b]	52.57	1.91[c]	3.65[c]
SEM[6]	53.9	0.105	0.098	0.062	0.724	0.037	0.102	0.131	0.109	0.709	0.033	0.095
F-ratio	0.68	816.29	2.72	4.74	8.70	170.73	121.25	3.08	4.19	1.30	138.48	131.83
P-value	0.642	<0.01	0.030	<0.01	<0.01	<0.01	<0.01	0.017	<0.01	0.279	<0.01	<0.01
Exp 2												
Control	2,954	18.6[a]	4.80	2.39[a]	49.80[a]	3.89[a]	7.81[a]	6.42	3.30	51.43[a]	2.91[a]	5.65[a]
UMD	2,904	14.2[b]	4.62	2.23[bc]	48.34[cd]	3.09[b]	6.40[b]	6.19	3.12	50.43[ab]	2.30[b]	4.57[b]
UMD + PHY	2,912	11.1[e]	4.73	2.32[ab]	49.16[ab]	2.35[d]	4.77[d]	6.02	3.06	50.97[a]	1.91[d]	3.74[d]
UMD + PHY + 25D	2,913	9.2[f]	4.68	2.29[b]	48.94[bc]	1.96[e]	4.01[e]	6.38	3.18	49.82[b]	1.44[e]	2.89[e]
Control + PHY	2,920	13.2[c]	4.67	2.29[b]	49.00[bc]	2.83[c]	5.77[c]	6.21	3.18	51.23[a]	2.13[bc]	4.15[c]
NC	2,837	12.6[d]	4.54	2.18[c]	48.00[d]	2.77[c]	5.77[c]	6.05	3.05	50.65[ab]	2.08[cd]	4.13[c]
SEM	39.4	0.136	0.133	0.043	0.681	0.069	0.096	0.229	0.091	0.757	0.089	0.141
F-ratio	1.42	724.33	1.90	4.58	5.98	292.30	207.02	1.19	1.36	2.51	45.02	44.06
P-value	0.235	<0.01	0.113	<0.01	<0.01	<0.01	<0.01	0.326	0.257	0.042	<0.01	<0.01
Exp 3												
Control	3,042	18.2[a]	4.90	2.41[a]	49.35[a]	3.72[a]	7.54[a]	6.57	3.41[a]	51.97[a]	2.78[a]	5.36[a]
UMD	2,999	13.6[b]	4.69	2.28[b]	48.65[ab]	2.91[b]	5.99[b]	6.32	3.24[ab]	51.45[a]	2.17[b]	4.22[b]
UMD + PHY	2,997	11.0[c]	4.79	2.30[ab]	48.00[b]	2.30[c]	4.80[d]	6.26	3.14[b]	50.18[b]	1.77[c]	3.52[c]
UMD + PHY + 25D	2,971	8.7[e]	4.71	2.28[b]	48.28[b]	1.84[d]	3.80[e]	6.44	3.23[ab]	50.06[b]	1.34[d]	2.68[d]
Control + PHY	3,009	13.5[b]	4.75	2.29[b]	48.18[b]	2.83[b]	5.89[b]	6.31	3.16[b]	49.98[b]	2.14[b]	4.29[b]
NC	2,957	10.6[d]	4.55	2.09[c]	45.81[c]	2.33[c]	5.10[c]	6.09	2.93[c]	48.06[c]	1.75[c]	3.64[c]
SEM	41.4	0.131	0.100	0.052	0.349	0.041	0.096	0.143	0.076	0.399	0.043	0.088
F-ratio	0.83	1,770.07	2.19	6.81	12.35	248.23	173.68	2.02	5.11	11.89	130.86	103.35
P-value	0.533	<0.01	0.071	<0.01	<0.01	<0.01	<0.01	0.221	<0.01	<0.01	<0.01	<0.01

[a–e]Values in a column with different superscript letters differ ($P \leq 0.05$).

[1]National Research Council (NRC) NPP recommendations; University of Maryland (UMD) NPP recommendations; UMD + phytase (PHY), UMD NPP concentrations reduced by 0.064% NPP plus 600 U of PHY/kg of diet; UMD + PHY + 25-hydroxycholecalciferol (25D), UMD NPP concentrations reduced by 0.090% NPP plus 600 units (U) of PHY and 70 μg of 25D/kg of diet; control + PHY, mimicked the industry practice of reducing NRC concentrations by 0.1% when PHY is added; and negative control (NC) that had 90% UMD NPP concentrations, except in the finisher and withdrawal phases of Exp 3.

[2]Nonphytin P intake (NPPI) from hatch to 49 d, per bird, based determined NPP, pen consumption, and number of birds at 49 d of age within the pen.

[3]Dry defatted femur or tibia bone and ash weight in grams per bone and ash percentage, respectively.

[4]Ratio of NPPI to femur or tibia dry defatted bone or ash weight.

[5]Values are means of 9 replicate pens, except for NC that had 10 replicate pens. Each pen had 56 birds, of which 10 birds were randomly sampled for analysis in Exp 1 and 3 and 8 birds in Exp 2.

[6]Weighted average of the SEM.

less than those for femur (Table 2), and thus sensitivity of the femur measurements was greater.

Results of DXA analyses were similar to those of bone ash. Broilers fed control diets had greater BMC, BMD, TMC, and TMD than those of broilers fed the NC diets in all Exp, except BMD in Exp 2 (Table 3). As with ash measurements, BMD, TMC, and TMD of birds fed UMD + PHY diets were not different from those of broiler chickens fed the control + PHY diets. Unlike femur ash weight, BMC of broilers fed control diets were greater than those fed any of the other TRT diets, in Exp 1 and 3, except for broilers fed control + PHY diets (Exp 1). The intake of NPP per gram of BMD, BMC, TMD, or TMC was greatest for birds fed the control diets and least for those fed the UMD + PHY + 25D diets. For data derived by DXA, there was no consistently greater *F*-ratio by tissue (whole carcass or drum) or by type of measurement (mineral density or content) for any of the measurements taken (Table 3), and thus there is no tissue or measure that clearly provides higher sensitivity to bone mineralization changes using this methodology.

Processing Study

In the processing part of Exp 3, where 22 broilers per pen were randomly sampled and commercially processed, the incidence of bruising (back, breast, wings, or legs) or of broken wings and legs between dietary TRT was not different (Table 4). Live and hot carcass weights of broilers fed the NC diets were less than those of broilers fed the control, UMD, and UMD + PHY diets. No differences in hot carcass yield or carcass parts yield were detectable between TRT (Table 5). Similarly, no differences were noted in carcass part weights, except that broilers fed NC diets had lower leg weight than those fed other TRT. However, an effect of dietary TRT was

Table 3. Nonphytin P (NPP) intake, whole body bone mineral content (BMC), whole body bone mineral density (BMD), tibia mineral content (TMC), and tibia mineral density (TMD) of 49-d-old broilers, experiment (Exp) 1, 2, and 3

Treatment[1]	NPPI[2] (g)	BMC[3] (g)	BMD[3] (g/cm)	NPPI/BMC or BMD[4] (g/g)	NPPI/BMC or BMD[4] (g/g per cm)	TMC[3] (g)	TMD[3] (g/cm)	NPPI/TMC or TMD[4] (g/g)	NPPI/TMC or TMD[4] (g/g per cm)
Exp 1									
Control	16.0[a5]	47.2[a]	0.411[a]	0.34[a]	39.02[a]	3.37[a]	0.362[a]	4.75[a]	44.20[a]
UMD	12.5[b]	43.0[b]	0.405[a]	0.29[b]	30.85[b]	3.10[ab]	0.359[a]	4.03[b]	34.82[b]
UMD + PHY	10.4[d]	43.0[b]	0.401[ab]	0.24[d]	25.94[e]	3.20[ab]	0.359[a]	3.28[cd]	29.00[c]
UMD + PHY + 25D	9.6[e]	42.3[b]	0.399[ab]	0.23[d]	24.01[f]	3.16[ab]	0.357[a]	3.04[d]	26.95[d]
Control + PHY	12.3[b]	46.2[a]	0.410[a]	0.27[c]	29.99[c]	3.36[a]	0.361[a]	3.69[bcd]	34.08[b]
NC	11.2[c]	39.1[c]	0.390[b]	0.29[b]	28.46[d]	2.91[b]	0.332[b]	3.73[bc]	33.74[b]
SEM[6]	0.105	1.63	0.006	0.008	0.270	0.193	0.0072	0.203	0.495
F-ratio	816.29	7.49	8.98	54.36	461.48	4.26	3.80	33.66	336.48
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Exp 2									
Control	18.6[a]	42.2[a]	0.389	0.44[a]	47.95[a]	3.26[a]	0.356[a]	5.76[a]	52.28[a]
UMD	14.2[b]	39.9[ab]	0.385	0.36[ab]	37.02[b]	2.97[b]	0.350[ab]	4.81[b]	40.65[b]
UMD + PHY	11.1[e]	41.1[ab]	0.388	0.24[c]	28.34[e]	3.06[b]	0.349[b]	3.65[d]	31.80[e]
UMD + PHY + 25D	9.2[f]	38.4[abc]	0.380	0.24[c]	24.16[f]	2.97[b]	0.348[bc]	3.11[e]	26.39[f]
Control + PHY	13.2[c]	38.6[abc]	0.380	0.35[b]	34.75[c]	3.00[b]	0.348[bc]	4.44[c]	37.92[c]
NC	12.6[d]	36.2[c]	0.379	0.35[b]	33.21[d]	2.67[c]	0.342[c]	4.73[bc]	36.77[d]
SEM	0.136	1.29	0.005	0.011	0.507	0.083	0.0023	0.113	0.467
F-ratio	724.33	2.81	0.88	45.11	283.11	6.02	3.83	67.46	507.98
P value	<0.01	0.026	0.511	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Exp 3									
Control	18.2[a]	44.0[a]	0.390[a]	0.42[a]	46.69[a]	3.30[a]	0.360[a]	5.52[a]	50.55[a]
UMD	13.6[b]	38.9[b]	0.379[b]	0.35[b]	36.00[b]	3.19[b]	0.357[ab]	4.27[b]	38.26[b]
UMD + PHY	11.0[c]	39.9[b]	0.381[ab]	0.28[d]	28.90[c]	3.18[b]	0.354[ab]	3.50[c]	31.15[c]
UMD + PHY + 25D	8.7[e]	38.5[b]	0.379[b]	0.23[e]	22.82[d]	3.16[b]	0.358[ab]	2.74[d]	24.17[d]
Control + PHY	13.5[b]	40.0[b]	0.379[b]	0.34[bc]	25.50[b]	3.08[b]	0.352[b]	4.38[b]	38.35[b]
Control	10.6[d]	33.1[c]	0.365[c]	0.32[c]	29.04[c]	2.54[c]	0.339[c]	4.19[b]	31.24[c]
SEM	0.131	1.18	0.0043	0.010	0.629	0.076	0.0025	0.094	0.446
F-ratio	1,770.07	9.19	4.87	47.96	600.79	17.94	13.42	79.96	810.34
P-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[a–f]Values in a column with different superscript letters differ ($P \leq 0.05$).

[1]National Research Council (NRC) NPP recommendations; University of Maryland (UMD) NPP recommendations; UMD + phytase (PHY), UMD NPP concentrations reduced by 0.064% NPP plus 600 units (U) of PHY/kg diet; UMD + PHY + 25-hydroxycholecalciferol (25D), UMD NPP concentrations reduced by 0.090% NPP plus 600 U of PHY and 70 μg 25D/kg diet; control + PHY, mimicked the industry practice of reducing NRC concentrations by 0.1% when PHY is added; and negative control (NC) that had 90% UMD NPP concentrations, except in the finishing and withdrawal phases of Exp 3.

[2]Nonphytin P intake (NPPI) from hatch to 49 d, per bird, based on determined NPP, pen consumption, and number of birds at 49 d of age within the pen.

[3]The BMC and BMD of whole carcasses and TMC and TMD of tibia were determined by dual energy x-ray absorptiometry.

[4]The NPPI to BMC, BMD, TMC, or TMD was the ratio of NPPI to mineral content or density.

[5]Values are means of 9 replicate pens except for the NC that had 10 replicate pens. Each pen had 56 birds, of which 10 birds were randomly sampled for analysis in Exp 1 and 3 and 8 birds in Exp 2.

[6]Weighted average of the SEM.

observed for both femur and tibia ash of the processed birds (Table 6). Femur and tibia weight, ash weight, and ash percentage of broilers fed control diets were greater than of those fed the NC diets (Table 6). Femur weight, percentage of femur ash, tibia weight, tibia ash weight, and percentage of tibia ash were similar between broiler chickens fed the control and UMD diets. The only bone mineralization measure that was less in broilers fed the UMD diets as compared with those fed the control diets was femur ash weight. There were no differences in any of the bone mineralization measures for broilers fed the control diets as compared with those fed the UMD + PHY diets. Broilers fed control diets had the highest NPPI to femur dry weight or tibia dry weight and ash weight (Table 6). Broilers fed UMD + PHY + 25D diets had the lowest NPPI per gram of femur dry weight, tibia dry weight, or tibia ash weight, followed by broilers fed UMD + PHY diets.

P Consumption, Excretion, and Retention and Effect on Litter P

Cumulative over the 3 Exp, P consumption per bird (hatch to 49 d) and P excretion were highest in broilers fed the control diets and lowest in those fed the UMD + PHY and UMD + PHY + 25D diets (Table 7). The reduction in P excretion from the birds on the control diets to the birds on UMD + PHY + 25D was 9.11 g/bird. Retention of P was improved from 40.19% in control-fed broilers to 53.40 and 56.13% in broilers provided UMD + PHY and UMD + PHY + 25D, respectively.

There was no difference between TRT in initial or final litter weight (Table 7), but weight of litter dry matter increased almost 4-fold during the 3 Exp, even though no litter was added as top dress. Thus, the increase in litter weight was due to excreta dry matter deposited to the litter during the studies. Litter P concentration was

Table 4. Incidence (%) of broken wings and legs and bruised backs, breasts, wings, and legs following commercial catching and processing of 49-d-old broilers, experiment 3

	Broken		Bruised			
Treatment[1]	Wings	Legs[2]	Back	Breast	Wings	Legs
Control	5.00[3]	1.70	0.50	1.00	22.80	4.00
UMD	6.99	2.20	2.00	0.50	13.95	2.20
UMD + PHY	5.40	1.60	0.60	0.50	15.93	2.20
UMD + PHY + 25D	7.29	4.30	1.00	0.00	14.94	2.10
Control + PHY	7.49	3.20	1.00	1.00	15.93	6.99
NC	5.00	1.00	1.00	1.00	9.98	2.40
SEM[4]	2.00	1.40	0.80	0.60	3.30	1.40
P-value	0.88	0.58	0.72	0.74	0.20	0.09

[1]National Research Council (NRC) nonphytin P (NPP) recommendations; University of Maryland (UMD) NPP recommendations; UMD + phytase (PHY), UMD NPP concentrations reduced by 0.064% NPP plus 600 units (U) of PHY/kg diet; UMD + PHY + 25-hydroxycholecalciferol (25D), UMD NPP concentrations reduced by 0.090% NPP plus 600 U of PHY and 70 μg 25D/kg diet; control + PHY, mimicked the industry practice of reducing NRC concentrations by 0.1% when PHY is added; and negative control (NC) that had 90% UMD NPP concentrations, except in the finishing and withdrawal phases of Exp 3.

[2]Leg defined as both thigh and drumstick.

[3]Values are means of 9 replicate pens, except for NC that had 10 replicate pens; 22 birds per pen were randomly selected for processing. Percentage data were transformed using the arcsine function to satisfy the assumptions of normality and homogeneity of variances for statistical analysis; the values shown are inverse conversion of arcsine means to percentages.

[4]Weighted average of the SEM.

greatest in pens where broilers fed the control diets were raised and lowest in pens where broilers fed the UMD + PHY and UMD + PHY + 25D diets were raised (Table 8). The greatest increase in percentage of litter P above the P concentration of newly placed litter was during the first experiment, followed by the second and then by the third experiment Litter P concentration increased little during Exp 3. The quantity of WSP in litter was greatest in pens where broilers were fed the control diets, followed by those fed the control + PHY for all Exp (Table 8). The lowest litter WSP concentration, in Exp 1 and 2, was observed in pens where broilers were fed the UMD, UMD + PHY, UMD + PHY + 25D, and NC diet (Table 8). By the end of Exp 3, litter WSP concentration was least in pens where broilers were fed the UMD + PHY + 25D and UMD + PHY diets.

DISCUSSION

Sensitivity of Bone and Mineralization Methodologies to Changes in Bone Mineralization

Femur and tibia mineralization were determined in these Exp using different methodologies. This was done to determine which bone and which method resulted in the greatest sensitivity to changes in mineralization. In these Exp, the *F*-ratios of femur measurements were greater than those of the tibia, except for femur weight in Exp 1. These greater *F*-ratios for femur mineralization measures allowed for greater sensitivity and thus for greater separation of TRT effects than was possible based on tibia mineralization measures. The *F*-ratios indicated that BMC was more sensitive than BMD. Selective scanning of tibia increased precision compared with whole-carcass scanning, in Exp 1 and 3, which was indicated by the higher *F*-ratios in TMC vs. BMC. In comparing the *F*-ratios of DXA (TMC) and femur ash weight, it is clear that greater separation of TRT is possible with TMC than ash weight. These results agree with those of Akpe et al. (1987), who reported that 3-point densitometry measurements had a higher *F*-ratio than bone ash percentage. Overall, the results from DXA measurements are consistent with those of the bone ash measurements and are in agreement with those of Akpe et al. (1987) and Mitchell et al. (1997), who reported that ash percentage and BMC are correlated.

Impact of Dietary TRT on Bone Mineralization

Femur ash weight of broilers fed control diets was greater in all Exp than that of broilers fed the UMD diets, which was not the case for tibia ash weight. However, it has been reported previously that femur ash in the latter phases of growth is a better indicator of bone mineralization status (Moran and Todd, 1994; Chen and Moran, 1995; Dhandu and Angel, 2003). The effect on femur ash is contrary to what had been found in previous battery pen studies (Angel et al., 2000a,b; Ling et al., 2000; Dhandu and Angel, 2003). These researchers determined P requirements for male Ross 308 broilers based on the response criterion that no differences in bone ash would be observed when diets were compared with an industry control diet, with no margin of safety built into the requirement. This discrepancy in results can be attributed at least in part to the lower determined NPP concentration in most UMD diets compared with the formulated concentrations in all the Exp of the current study. This difference resulted in broilers fed the UMD diets consuming less NPP than the required concentration, resulting in decreased femur ash weight.

Table 5. Live weight, hot carcass weight, and weight and yield of carcass components of processed 49-d-old broilers, experiment 3

Treatment[1]	BW[2] (g)	Hot carcass Weight[4] (g)	Hot carcass Yield[5] (%)	Carcass component weight (g) Breast[6]	Wings[7]	Legs[8]	Barrel[9]	Carcass component yield[3] (%) Breast	Wings	Legs	Barrel
Control	2,916[a10]	2,091[a]	71.71	525	269	665[a]	637	25.64	12.56	32.71	29.75
UMD	2,890[ab]	2,072[ab]	71.68	523	269	666[a]	622	25.78	12.59	31.36	29.26
UMD + PHY	2,920[a]	2,098[a]	71.87	536	272	671[a]	622	26.09	12.65	31.70	28.87
UMD + PHY + 25D	2,849[bc]	2,045[bc]	71.77	511	266	657[a]	619	25.58	12.68	31.36	29.50
Control + PHY	2,867[b]	2,070[ab]	72.20	530	266	666[a]	616	26.17	12.55	31.33	28.94
NC	2,816[c]	2,018[c]	71.67	509	267	636[b]	613	25.85	12.82	30.68	29.51
SEM[11]	26.0	19.7	0.246	1.2	2.5	7.8	8.0	0.380	0.157	0.68	0.35
P-value	<0.01	<0.01	0.408	0.097	0.492	<0.01	0.356	0.763	0.515	0.434	0.399

[a–c]Values in a column with different superscript letters differ ($P \leq 0.05$).

[1]National Research Council (NRC) nonphytin P (NPP) recommendations; University of Maryland (UMD) NPP recommendations; UMD + phytase (PHY), UMD NPP concentrations reduced by 0.064% NPP plus 600 units (U) of PHY/kg diet; UMD + PHY + 25-hydroxycholecalciferol (25D), UMD NPP concentrations reduced by 0.090% NPP plus 600 U of PHY and 70 μg 25D/kg diet; control + PHY, mimicked the industry practice of reducing NRC concentrations by 0.1% when PHY is added; and negative control (NC) that had 90% UMD NPP concentrations, except in the finishing and withdrawal phases of Exp 3.

[2]Farm BW with no feed withdrawal.

[3]Percentage of yield, hot carcass weight basis.

[4]Hot carcass weight taken just prior to chill tank.

[5]Hot carcass as a percentage of full-farm BW.

[6]Weight of both pectoralis major and minor (butterfly cut) with skin, removed by processing plant deboning personnel.

[7]Wing includes humerus, radio-ulna, and metacarpals.

[8]Leg defined as thigh and drumstick.

[9]Barrel defined as rib cage and backbone.

[10]Values are means of 9 replicate pens except for NC that had 10 replicate pens; 22 birds per pen were randomly selected for processing.

[11]Weighted average of the SEM.

In contrast, the NPP equivalency of PHY as determined in battery pens (Angel et al., 2001) could be applied directly since no difference in the ash weight of broilers fed the control or UMD + PHY diet was detected. Lack of differences in the femur and tibia ash weight of broilers fed the control + PHY or UMD + PHY diets indicates that excess dietary NPP was provided to broilers fed control + PHY diet. The fact that excess NPP was consumed by broilers fed the control + PHY is evident in the higher NPPI to femur ash weight ratio and in the P and WSP concentrations in litter after 3 trials as compared with those of broilers fed the UMD + PHY. Broilers fed control diets consumed almost 1 g more NPP per gram of femur ash weight compared with those fed UMD + PHY diets. Broilers fed UMD + PHY + 25D diets consumed the lowest NPP per gram of ash weight, yet ash measurements were

Table 6. Bone measurements of 49-d-old broilers processed in a commercial processing plant, experiment 3

Treatment[1]	BW (g)	NPPI[2] (g)	Femur[3] Weight (g)	Femur[3] Ash (g)	Femur[3] Ash (%)	NPPI/femur[4] Weight (g/g)	NPPI/femur[4] Ash (g/g)	Tibia[3] Weight (g)	Tibia[3] Ash (g)	Tibia[3] Ash (%)	NPPI/tibia[4] Weight (g/g)	NPPI/tibia[4] Ash (g/g)
Control	2,916[a5]	18.19[a]	4.97[a]	2.31[a]	46.51[a]	3.66[a]	7.87[a]	6.68[ab]	3.29[a]	49.25[a]	2.73[a]	5.53[a]
UMD	2,890[ab]	13.63[b]	4.83[ab]	2.22[b]	46.16[a]	2.82[b]	6.13[b]	6.54[ab]	3.19[ab]	48.69[a]	2.09[b]	4.28[b]
UMD + PHY	2,920[a]	11.01[c]	4.94[a]	2.26[ab]	45.89[a]	2.23[d]	4.87[d]	6.75[a]	3.27[a]	48.53[a]	1.63[d]	3.38[d]
UMD + PHY + 25D	2,849[bc]	8.65[e]	4.84[ab]	2.19[b]	46.15[a]	1.78[e]	3.95[e]	6.42[b]	3.12[b]	48.74[a]	1.35[e]	2.77[e]
Control + PHY	2,867[b]	13.45[b]	4.80[bc]	2.23[ab]	46.53[a]	2.81[b]	6.04[b]	6.45[b]	3.16[b]	49.13[a]	2.09[b]	4.26[b]
NC	2,816[c]	10.60[d]	4.44[c]	1.93[c]	43.35[b]	2.38[c]	5.48[c]	6.07[c]	2.80[c]	46.11[b]	1.75[c]	3.79[c]
SEM[6]	26.0	0.131	0.101	0.033	0.421	0.039	0.069	0.092	0.045	0.257	0.023	0.046
P-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[a–e]Values in a column with different superscript letters differ ($P \leq 0.05$).

[1]National Research Council (NRC) nonphytin P (NPP) recommendations; University of Maryland (UMD) NPP recommendations; UMD + phytase (PHY), UMD NPP concentrations reduced by 0.064% NPP plus 600 units (U) of PHY/kg diet; UMD + PHY + 25-hydroxycholecalciferol (25D), UMD NPP concentrations reduced by 0.090% NPP plus 600 U of PHY and 70 μg 25D/kg diet; control + PHY, mimicked the industry practice of reducing NRC concentrations by 0.1% when PHY is added; and negative control (NC) that had 90% UMD NPP concentrations, except in the finishing and withdrawal phases of Exp 3.

[2]Nonphytin P intake (NPPI) from hatch to 49 d, per bird, based on determined NPP, pen consumption, and number of birds at 49 d of age within the pen.

[3]Dry defatted femur or tibia weight and respective ash weight and ash percentage.

[4]Ratio of NPPI to femur or tibia dry defatted weight or ash weight.

[5]Values are means of 9 replicate pens, except for the NC that had 10 replicate pens; 22 birds per pen were randomly selected for processing.

[6]Weighted average of the SEM.

Table 7. Cumulative consumed, excreted, and retained P; litter water-soluble P (WSP); and initial and final litter weights on a dry matter basis over experiments (Exp) 1, 2, and 3

Treatment[1]	Consumed P[2] (g/bird)	Initial litter weight[3] (kg)	Final litter weight[3] (kg)	Excreted P[4] (g/bird)	Retained P[5] (%)	Final litter WSP[6] (g/bird)
Control	32.71[a7]	59.17	217.02	19.55[a]	40.19[c]	7.66[a]
UMD	28.51[b]	59.53	212.19	14.89[b]	47.75[b]	3.92[c]
UMD + PHY	25.17[e]	60.10	205.44	12.00[d]	53.40[a]	3.17[de]
UMD + PHY + 25D	23.81[f]	59.73	209.87	10.44[e]	56.13[a]	2.84[e]
Control + PHY	27.74[c]	59.86	212.88	14.47[bc]	47.82[b]	6.28[b]
NC	26.52[d]	59.33	214.11	13.78[c]	48.00[b]	3.60[cd]
SEM[8]	0.151	1.901	3.728	0.316	1.252	0.152
P	<0.01	0.839	0.262	<0.01	<0.01	<0.01

[a–f]Values in a column with different superscript letters differ ($P \leq 0.05$).

[1]National Research Council (NRC) nonphytin P (NPP) recommendations; University of Maryland (UMD) NPP recommendations; UMD + phytase (PHY), UMD NPP concentrations reduced by 0.064% NPP plus 600 units (U) of PHY/kg diet; UMD + PHY + 25-hydroxycholecalciferol (25D), UMD NPP concentrations reduced by 0.090% NPP plus 600 U of PHY and 70 μg 25D/kg diet; control + PHY, mimicked the industry practice of reducing NRC concentrations by 0.1% when PHY is added; and negative control (NC) that had 90% UMD NPP concentrations, except in the finishing and withdrawal phases of Exp 3.

[2]Total P consumed for the 3 Exp.

[3]Clean pine shavings weight at the start of Exp 1 and weight of built-up litter at the end of Exp 3. Concentration of P in clean litter at the start of Exp 1 was 0.007%.

[4]Based on P in litter at the end of Exp 3 − P in litter at the start of Exp 1 ÷ the number of birds that were finished over the 3 Exp, by pen.

[5]Percentage P retained, calculated based on P consumed (from analyzed diet P), and P excreted for the 3 Exp.

[6]Based on WSP concentration in litter and weight of litter at the end of Exp 3.

[7]Values are means of 9 replicate pens, except for NC that had 10 replicate pens.

[8]Weighted average of the SEM.

similar to those of broilers fed UMD + PHY or control + PHY, indicating that 25D spared some of the dietary NPP. Compared with those fed UMD + PHY diets, broilers fed UMD + PHY + 25D diets consumed 0.80, 1.91, and 2.36 g and 2.69, 4.00, and 4.80 g less NPP from hatch to 49 d, respectively, in Exp 1, 2, and 3 as compared with control + PHY-fed birds. These results indicate that 25D and PHY were acting synergistically. Others have reported additive effects of vitamin D metabolites and PHY in broilers (Biehl et al., 1995; Angel et al., 2001) and turkeys (Angel et al., 2002).

Impact of Dietary TRT on Processing Yields

Even though there were differences in P and NPPI as well as bone ash weight and density measurements in Exp 3, no differences in carcass yield or in the incidence of bruised parts or broken legs and wings were observed at processing. These results are contrary to those reported by Moran and Todd (1994). In their work, drumstick bruising ($P \leq 0.06$) and deformation of drumsticks ($P \leq 0.05$) was increased as a result of feeding a low-P diet.

Table 8. Phosphorus and water-soluble P (WSP) in litter (dry matter basis) at the end of each experiment (Exp) 1, 2, and 3

Treatment[1]	Exp 1 P[2] (%)	Exp 2 P (%)	Exp 3 P (%)	Exp 1 litter WSP[3], (%)	Exp 2 litter WSP, (%)	Exp 3 litter WSP, (%)	Exp 1 WSP/P[4] (%)	Exp 2 WSP/P (%)	Exp 3 WSP/P (%)
Control	1.04[a5]	1.35[a]	1.51[a]	0.37[a]	0.47[a]	0.55[a]	34.44[a]	34.88[a]	35.26[b]
UMD	0.88[b]	1.12[b]	1.18[b]	0.19[c]	0.24[c]	0.29[c]	21.56[bc]	21.79[bc]	23.64[c]
UMD + PHY	0.75[c]	0.95[d]	0.97[d]	0.18[c]	0.24[c]	0.24[de]	23.94[b]	24.96[bc]	24.16[c]
UMD + PHY + 25D	0.71[c]	0.86[e]	0.87[e]	0.17[c]	0.22[c]	0.21[e]	24.46[b]	25.08[b]	24.05[c]
Control + PHY	0.86[b]	1.09[b]	1.17[b]	0.27[b]	0.42[b]	0.46[b]	31.85[a]	38.26[a]	40.42[a]
NC	0.87[b]	1.04[c]	1.06[c]	0.16[c]	0.21[c]	0.26[cd]	18.52[c]	20.19[c]	22.95[c]
SEM[6]	0.017	0.020	0.020	0.010	0.015	0.013	1.06	1.19	1.10
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[a–e]Values in a column with different superscript letters differ ($P \leq 0.05$).

[1]National Research Council (NRC) nonphytin P (NPP) recommendations; University of Maryland (UMD) NPP recommendations; UMD + phytase (PHY), UMD NPP concentrations reduced by 0.064% NPP plus 600 units (U) of PHY/kg diet; UMD + PHY + 25-hydroxycholecalciferol (25D), UMD NPP concentrations reduced by 0.090% NPP plus 600 U of PHY and 70 μg 25D/kg diet; control + PHY, mimicked the industry practice of reducing NRC concentrations by 0.1% when PHY is added; and negative control (NC) that had 90% UMD NPP concentrations, except in the finishing and withdrawal phases of Exp 3.

[2]Concentration of P in litter at the end of Exp 1, 2, and 3. Concentration of P in clean litter at the start of Exp 1 was 0.007%.

[3]Concentration of WSP in the litter at the end of Exp 1, 2, and 3. Concentration of WSP in clean litter at the start of Exp 1 was undetectable.

[4]Proportion of P as WSP, expressed as a percentage.

[5]Values are means of 9 replicate pens, except for NC that had 10 replicate pens.

[6]Weighted average of the SEM.

However, key differences exist between the 2 studies. In the Moran and Todd (1994) study, 3 age phases were used with broilers on the control and low-P diets fed, calculated, available P concentrations of 0.40, 0.35, and 0.31% on the control diets and 0.31, 0.28, and 0.25% on the low-P diets in the starter (hatch to 21 d), grower (21 to 42 d) and finisher (42 to 56 d) phases, respectively. Available P concentrations for the control diets were higher than those fed in the current study, but those in the low-P starter phase were much lower than those in the current study. This early severe deficiency can result in deficiency signs later in the growth phase (Persia et al., 2005). Moreover, the 56-d BW of the NC in the Moran and Todd (1994) research was 2,740 g, whereas the 49-d BW of the processed birds in the current research was 2,816 g. Overall, broilers in the Chen and Moran (1995) research consumed more NPP over a longer period and gained less weight compared with the NC birds in the current research.

There was no effect of the different TRT on processing yield of the body parts measured. These results are in agreement with those of Moran and Todd (1994), who reported no differences in the chilled carcass weight. Percentage yields of the different parts in the current study are in agreement with those reported by Moran and Todd (1994) and Chen and Moran (1995).

Tibia and femur ash of processed birds were analyzed to test the effect of stress of processing (catching, transportation, stunning, scalding, picking, and evisceration). Even though there was no effect of TRT on visual carcass inspection or on percentage parts yields, there were TRT differences in the femur and tibia measurements of the processed birds. The ash results were similar to the ash and DXA measurements of broilers that were not sent to the processing plant.

Impact of Dietary TRT on Litter Weight, P, and WSP

There was a 3.5-fold increase in dry matter weight of litter between the start of Exp 1 and the end of Exp 3. The only possible contributor to this increase in weight is the excreta itself. Given this weight increase and its source, the pine shavings accounted for approximately 27% of the weight of the litter by the end of the third flock. Concentration of P in litter was similar after Exp 2 and 3 in all TRT except the control TRT. Once litter is constituted primarily by excreta, P concentration will not change greatly over time, making it more important to measure actual mass of P rather than the concentration of P in the excreta to determine effect on the environment.

Grams of P excreted by broilers, from hatch to 49 d of age (average BW of 2.9 kg/bird) fed UMD diets were less (14.89 g/bird) than that excreted by broilers fed the control diets (19.55 g/bird). The feeding requirement decreased excreted P by 4.66 g/bird, a reduction of nearly 24%. This result corroborates the findings of Waldroup et al. (2000) and Yan et al. (2000), who reported reductions of 25 to 28% in litter P when broilers were fed closer requirements. Further decreases in excreted P were achieved when the UMD + PHY and the UMD + PHY + 25D were imposed, resulting in decreases of 7.55 and 9.11 g/bird, respectively, as compared with birds fed the control diet. A maximum reduction of 46% in excreted P was observed when PHY and 25D were used.

Concentration of WSP was highest in litter from pens where broilers were fed excessive P or excessive P in the presence of PHY. These findings agree with those of Moore et al. (1998), Applegate et al. (2003), Maguire et al. (2003), and Angel et al. (2005a). Addition of PHY to diets containing an adequate or excessive amount of P resulted in higher WSP concentrations (0.46% WSP in litter after 3 flocks of broilers were fed the control + PHY) as compared with that of broilers fed at NPP requirements (0.29% WSP). Correct use of PHY resulted in reduced WSP concentration (0.29 vs. 0.24% WSP) after 3 flocks in the litter from pens where broilers were fed the UMD and UMD + PHY, respectively. These results agree with those of Moore et al. (1998), Applegate et al. (2003), Maguire et al. (2003), and Angel et al. (2005a) but are in contrast to those of DeLaune et al. (2001). Differences in methodologies and excreta and litter handling prior to analysis appear to account for this discrepancy (Angel et al., 2005a).

Overall, the UMD NPP requirements should be used with care, and safety margins must be applied by industry nutritionists based on their specific ingredient nutrient formulation matrix accuracy and broiler production management limitations. The addition of PHY and 25D with the appropriate reduction in dietary NPP results in reduced feed and litter P without any deleterious affects on performance or processing yields in broilers. Consistently in these 3 Exp, correct use of PHY resulted in reductions in litter WSP concentration and no change in the amount of P constituted by WSP. Feeding of appropriately reduced NPP concentrations with the addition of PHY resulted in bone mineralization that was not different than that of birds fed UMD-recommended concentrations. Further reductions in diet NPP beyond the current industry practice of reducing NRC (1994) NPP concentrations by 0.1% when PHY is included in the diet can be made without affecting the bone mineralization. Finally, processing work shows that there is no effect of small reductions in bone mineralization, as compared with NRC (1994)-recommended concentrations, on condemnations or losses at the processing plant. Processing results from the current work show that bones need not be fully mineralized to prevent condemnations at processing. Future work needs to be conducted to determine at what concentration of dietary NPP there would be increased condemnations at processing.

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